LIPID BINDING IN WHEATS AND IN FLOURS VARYING WIDELY IN BREAD-MAKING POTENTIALITIES

by

CHIEN-MEI CHIU

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INTRODUCTION

Lipids are a minor constituent of most cereals. Wheat contains 2-4%, and wheat flour 1-2% lipids. Wheat lipids were for many years an unappreciated factor governing flour quality, because of the lack of suitable methods to isolate, fractionate and identify the numerous, complex, and labile lipid components.

Recently, there have been developed several techniques suitable for the investigation of that complex group of substances. Available information on lipids in wheat and in bread-making has shown the lipids to play a much more important role than has been previously realized.

The effects of lipid binding in bread-making were studied by researchers in England, Canada, Australia, and the U. S. A. Lipids readily extractable from flour are partly bound when the flour is made into a dough. Further binding was found when the dough was made into bread. Little is known about lipid binding in different varieties of wheat during bread-making. This study deals with lipid binding in kernels of varying size, changes in extractability of lipids during baking of bread from various dough formulations, and comparison of the effects of lipids on bread-making of flours milled from various wheat classes and varieties.

REVIEW OF LITERATURE

The role of lipids in bread-making was reviewed very briefly by Swanson (1937). Earlier work on lipids in wheat and flour up to 1944 has been reviewed by Bailey. That review gives comprehensive lists of lipids in wheat and various wheat products. The important glycolipids, which were only recently found in cereals, are not listed. No attempt has been made to correlate lipid contents with bread-making quality. Similarly, Kent-Jones and Amos (1947) devote only a few lines in their book to wheat flour lipids. The most recent monograph of the American Association of Cereal Chemists (Mecham, 1964) shows clearly the large advances which were made in recent years in studies of cereal lipids. The advances were made possible by availability of new analytical techniques, such as gas-liquid chromatography (GLC), silicic acid column chromatography, countercurrent distribution, and thin-layer chromatography (TLC).

Lipids. Lipids are characterized by their sparing solubility in water and considerable solubility in organic solvents, physical properties which reflect their hydrophobic, hydrocarbon nature (Conn and Stumpf, 1964). Lipids, a rather heterogeneous class of compounds, are traditionally classified as (a) simple lipids, (b) compound lipids, and (c) derived lipids. All classes are highly heterogenous.

In wheat, lipids form 1-2% of the endosperm, 8-15% of the germ, and about 6% of the bran, with an average of 2-4% of the whole kernel (Fisher, 1962). The amounts and kinds of lipids

in wheat flour depend on many factors, such as the type of flour and the grist used (Sullivan and Near, 1927), the milling extraction (Sullivan and Near, 1928), the age of the flour (Greer, et al., 1954), the kind of solvent used to extract the lipids (Cookson and Coppock, 1956), and the stage of maturation of the wheat (Daftary and Pomeranz, 1965a). In wheat and flour, simple lipids contain mainly triglycerides and small amounts of mono- and di-glycerides; compound lipids contain primarily glycolipids and phospholipids; derived lipids contain mainly free fatty acids. In addition, there are some other compounds soluble in fat solvents, such as tocopherols and unsaponifiable material.

Lipids in Wheat and Milled Wheat Products. The wheat kernel contains about 3% of lipids which are not uniformly distributed throughout the kernel. Sullivan and Near (1928) studied lipids in milling products of a hard red spring wheat. More lipids were extracted with an alcohol-ether mixture than with ether alone. The difference was larger in flours than in bran, shorts, and germ. Total lipid contents were 3.02, 1.79-4.39, 5.74, 7.29, and 12.04% in whole wheat, various flours, bran, shorts, and germ, respectively. The highest percentage of phosphorus was found in the lipids of the highest grade of flour (patent) which contained the least total phosphorus. The phosphorus content of the lipids decreased with decreasing refinement and increasing total phosphorus of the products from which they were extracted. The nitrogen content of the lipid extracted from patent flour was higher than of that extracted from

the germ.

Shollenberger et al., (1949) found that petroleum-ether extracted 1.37-2.56% lipids from 481 samples of wheat from five crop years and 63 locations in the United States and Canada. They found that amount of petroleum-ether-extractable lipids, unlike test weight, ash, protein, sugar, and starch, was a varietal characteristic as grain of comparably grown varieties differed more than grain of the same variety grown under different conditions.

Mason and Johnston (1958) studied lipids from two hard red winter wheat flours, Ponca and Red Chief, differing in bread-baking quality. The lipids were extracted with water-saturated butanol and fractionated to study compositional differences. Phosphatide fractions from each of the flours were subjected to 800 transfer countercurrent distributions, using the solvent system water : methanol : water-saturated butanol : 1-heptane (3:17:40:60). Red Chief flour lipids contained more choline phospholipids and less phosphatidyl ethanolamine than Ponca flour lipids. It was suggested that the differences might be related to the poor and good baking quality of flour from Red Chief and Ponca wheat, respectively.

Nelson et al., (1963a) extracted lipids from whole-wheat meal with water-saturated 1-butanol and chromatographed the extract on silicic acid columns. With a gradient elution solvent system, of increasing concentrations of ethyl-ether in petroleum-ether followed by absolute methanol, wheat lipids were separated

into four fractions of increasing polarity. Fraction I presumably contained hydrocarbons and steryl esters; fraction II consisted almost entirely of triglycerides; fraction III was very heterogenous and contained among other compounds, the bulk of wheat pigments; and fraction IV, which was rich in phosphorus, nitrogen, and sugars, was presumed to consist largely of phospholipids and glycolipids. Wheat lipids extractable by water-saturated 1-butanol but not by ethyl-ether consist almost entirely of polar material as distinguished from the predominantly nonpolar nature of the ethyl ether-extractable substances. Fractionation of n-butyl alcohol extracts from bran, germ, and first middlings streams from a commercial mill indicated marked differences in the lipids of various wheat tissues.

Nelson et al., (1963b) analyzed the fatty acids of the triglycerides and the total fatty acids of wheat, bran, germ, and endosperm by countercurrent distribution and gas-liquid chromatopgraphy. Relatively few types of triglycerides were found to be present. The total as well as triglyceride fatty acids present in bran, germ, and endosperm are quite similar although differences do exist.

Fisher et al., (1964) examined lipid content and composition of flours of 70% extraction milled from 5 wheat varieties grown in the United States and of two varieties grown in Britain. Varietal, seasonal, and environmental differences were demonstrated. Thin-layer chromatography showed no qualitative differences in the lipid composition of the seven varieties.

Di-glycerides (1, 2- and 1, 3-) were separated and identified among the less polar lipids, and lysolecithin among the polar lipids. Two-dimensional thin-layer chromatography of flour lipids revealed 23 components including phosphatidyl choline, phosphatidyl ethanolamine, glycerophosphoryl inositol, and glycerophosphoryl glycerol.

The lipid content of hard red spring wheats was higher than that of hard red winter wheats. The U. S. wheats showed significant varietal differences in lipid-N and lipid-P, but the differences were not consistently correlated with bread-making strength. No significant and consistent correlations could be established between loaf volume and choline, phosphatidyl inositol, total glycolipid, or fatty acid distribution (bound and free).

McKillican and Sims (1964) prepared flour samples from intact and degermed kernels of hard red spring, soft white spring, and amber durum wheats. All three varieties of wheat had the same distribution pattern of polar lipids in free lipid. The distribution of lipid classes in bound lipid differed from that in free lipid. However, the patterns obtained for each type of wheat resembled each other; the galactolipids were the major component and phosphatidyl choline the dominant phospholipid.

The dominant fatty acid component of the free lipid triglycerides, linoleic acid, was present to the extent of over 50%. The other major fatty acids of this fraction were palmitic and oleic. The ratio of total saturated to total unsaturated

fatty acids was the same in all varieties. In durum, the ratio of palmitic to oleic acid was lower than of oleic to linoleic; in the other wheats the ratio was reversed. Fatty acids of polar free lipids were much more saturated than fatty acids of free triglycerides. Fatty acids of bound polar lipids resembled fatty acids of free polar lipids. Lipids from degermed flour had less linoleic acid than the lipids from intact grain.

McKillican (1964) isolated the phospholipid-glycolipid mixture from the "free" (hexane soluble) and "bound" (hexane insoluble, water-saturated butanol extractable) lipids of wheat endosperm. The mixture was fractionated by column and thin-layer chromatography. Similar patterns were observed in all three wheat varieties. The main components of the phospholipid-glycolipid mixture were digalactosyl glyceride, monogalactosyl glyceride, and phosphatidyl choline. The sterol-containing glycolipids were also present in appreciable quantities. A complex, unidentified mixture of low R_f material was encountered in the bound lipids. Differences in the fatty acid composition of both free and bound lipid were observed between spring and durum wheat. The fatty acids of the phospholipids and glycolipids were mainly linoleic, palmitic, and oleic. The most highly unsaturated components were the galactolipids. Fatty acids of phosphatidyl choline were more unsaturated than of lysophosphatidyl choline.

Burkwall and Glass (1965) studied the fatty acids of the free (petroleum-ether-extractable) and of the bound (extracted

by water-saturated butanol following petroleum-ether) lipids of whole wheat and its experimentally-milled fractions by gas-liquid chromatography. The total fatty acid content of the free lipids was approximately twice that of the bound lipids and ranged from 77 to 92% in the former and from 41 to 53% in the latter. In all fractions, linoleic acid predominated and was followed by palmitic, oleic, linolenic, and stearic acids in decreasing order. Straight-grade flour contained considerably more linoleic and palmitic acids and less oleic and linolenic acids than did the bran and the shorts. Calculated iodine values were virtually identical for the lipids of all three fractions.

Pomeranz and Chung (1965) extracted lipids from single wheat kernels and fractionated the extracts by thin-layer chromatography into 7-8 nonpolar and 8-9 polar components. In addition, the lipids in half a wheat kernel, and in the bran, endosperm, and germ of the other half were fractionated and quantitated by optical density measurements of sulfuric acid-charred spots. The highest concentrations of triglycerides was found in the germ, the lowest in the endosperm. The germ had the highest concentration of phospholipids, the endosperm was rich in mono- and digalactosyl glycerides.

Stevan and Houston (1966) studied five hard red spring and five hard red winter flours. Lipids were extracted with water-saturated butanol and washed with a dilute solution of calcium chloride. Lipid contents of total hard red spring and hard red winter flours varied significantly and averaged respectively

1.54% and 1.30%. The ratios of nonpolar to polar fractions were higher in hard red spring than in hard red winter flour lipids. Total lipids and the nonpolar to polar ratio showed a positive correlation of r = 0.73. The polar lipid contents of HRS and HRW flours were similar and ranged from 0.61 to 0.75%. The correlation of polar with total lipids was low, with r = 0.13. The contents of nonpolar lipids of the flours varied widely and ranged from 0.50 to 1.04% of dry flour. The correlation between nonpolar and total lipid contents was high, with r = 0.93.

Pomeranz et al., (1966a) extracted lipids with petroleum-ether and with water-saturated n-butanol from flours milled from 8 composite hard red winter, 5 hard red spring, and one each from soft red, durum, and club wheat varieties. The butanol-extracted lipids were fractionated into nonpolar and polar lipids by silicic acid column chromatography, and the two major fractions were subfractionated by thin-layer chromatography. The extracted, washed lipids contained about 52% nonpolar and 48% polar lipids. Flour milled from durum wheat contained substantially less polar lipids than flours milled from hard red winter or hard red spring wheats. The triglycerides constituted about 50% of the nonpolar lipids. Among the polar lipids, digalactosyl glyceride was the major component (about 40%); an unidentified compound, and a mixture of monogalactosyl glyceride with phosphatidic acid constituted about 20% each; and phosphatidyl ethanolamine, phosphatidyl choline, and phosphatidyl serine comprised about 4, 7, and 4.5% of the polar lipids, respectively.

In a later study (Pomeranz et al., 1966b), lipids were extracted with petroleum-ether and with water-saturated n-butanol from 8 hard red winter, 5 hard red spring, and one each from soft red, durum, and club wheat varieties from 2 harvests. Durum wheats contained the highest lipid contents, and the highest concentration of nonpolar lipids. The bread-making wheat varieties had a lipid content which was consistent for the 2 years examined. The total and nonpolar lipid contents of hard red spring wheats were higher than those of hard red winter wheats. The polar lipid contents were substantially higher in wheats than in flours milled from the wheats. Nonpolar lipids constituted about one-half of the flour lipids and two-thirds of the wheat lipids. Concentrations of triglycerides were higher in wheat than in flour nonpolar lipids. Glycolipids were present in comparable concentrations in wheat and in flour polar lipids; concentration in polar lipids of phosphatidyl choline was higher and of other phospholipids was lower in wheat than in flour polar lipids.

Fisher et al., (1966) studied U. S. wheats and English-grown wheats harvested in 1960-1962. Total lipids were extracted by water-saturated butanol. The same lipid components were found in each of the varieties studied. English flours contained much larger amounts of unesterified sterols than U. S. varieties of the same crop year. Clear differences were found in fatty acid distributions; English samples contained higher amounts of linoleate than U. S. samples. Quantitative lipid analyses showed substantial varietal, seasonal, regional, and environmental

differences in lipid content and composition of the samples.

Role of Lipids in Wheat and in Bread-Making. Wheat flour lipids play an important role in oxidation, in storage deterioration, and in bread-making (Mecham and Pence, 1957; Glass, 1960, 1962; Fisher, 1962; Coppock and Daniels, 1962; Daniels, 1963; Mecham, 1964; and Pomeranz, 1966).

Smith and Andrews (1957) found that mixing of flour-water doughs is accompanied by uptake of oxygen. Smith et al., (1957) reported that polyunsaturated fatty acids in flour doughs are apparently oxidized by the action of lipoxidase. Tsen and Hlynka (1962) found by use of the thiobarbituric acid method that lipid peroxides were formed in dough during mixing in air or oxygen. Added lipoxidase increased the rate of peroxidation of flour lipids. On the other hand, antioxidants inhibited the peroxidation. A number of synthetic organic peroxides increased the structural relaxation constant of dough, indicating a similar improving role for flour lipid peroxides. When sulfhydryl-blocking reagents and improving oxidants were incorporated into dough, lipid peroxidation was increased. This suggested that flour lipids compete with sulfhydryl groups for available oxygen in dough. Free lipids were responsible for the peroxidation.

In a later study, Tsen and Hlynka (1963) showed that untreated flour dough lost its sulfhydryl groups faster than did defatted flour dough when mixed in oxygen or air for over 5 minutes. The difference between untreated flour dough and defatted flour dough was attributed to the oxidation of lipids. Oxidized

flour lipids or oxidized methyl linoleate when incorporated into doughs increased the sulfhydryl oxidation and exerted an improving effect (increased extensigram height), as did such simple peroxides as t-butyl hydroperoxide, methyl ethyl ketone peroxides, and acetone peroxides. It thus appeared that when enough oxygen is available in dough, it reacts with both sulfhydryl groups and lipids, and that oxidized lipids also oxidize sulfhydryl groups and thus exert an improving effect.

Deterioration of grain and cereal products in storage is accompanied by increased acidity, caused by the formation of free fatty acids, acid phosphates, and amino acids. Daftary and Pomeranz (1965b) followed changes in lipid composition during grain deterioration. Grain deterioration was accompanied by lowering of polar lipids and rapid disappearance of at least five ninhydrin- or Dragendorff-reagent positive polar lipids. The breakdown of polar lipids was more rapid and more intensive than formation of free fatty acids or disappearance of triglycerides.

The role of wheat flour lipid fractions in bread-making has been studied by many investigators. Coppock et al., (1954) added to dough 0.003% of an acetone-insoluble phosphatide fraction separated from the precipitate formed on aging of flour oil. The phosphatide improved substantially the loaf volume of bread baked from defatted flour. Lipids were fractionated by countercurrent distribution, the fractions were added to extracted flour, and bread was baked from doughs made from the

resulting flour with or without addition of lard. The fractions differed markedly in their effects on loaf volume and crumb texture.

Pomeranz et al., (1965) studied the effects of polar and nonpolar wheat flour lipids on quality of bread baked from untreated wheat flour. When the bread formula included 3 g vegetable shortening, adding 0.5 g of nonpolar, polar, or the unfractionated lipids from wheat flour had no significant effect on crumb grain and loaf volume. However, loaf volume of bread baked without vegetable shortening was increased strikingly by adding 0.5 g of polar flour lipids. Addition of 0.5 g of nonpolar lipids did not increase loaf volume significantly. Addition of 0.5 g of unfractionated flour lipids (approximately 1:1 ratio of polar and nonpolar) gave an intermediate volume, expected from the contribution of the lipid components.

In a later study, Pomeranz et al., (1966c) investigated the effects on loaf volume and bread characteristics of commercial vegetable shortening, and polar and nonpolar lipids isolated from 6 wheat flours varying widely in bread-making potentialities. The effects on bread quality of shortening or of polar lipids were found to be independent of wheat class or variety.

Daftary et al., (1967) extracted free (petroleum-ether-soluble) and bound (soluble in water-saturated-butanol, following petroleum-ether) lipids from a composite hard red winter wheat flour, from two other hard red winter wheat flours varying

widely in bread-making potentialities, and from a hard red spring wheat flour. The lipids were fractionated into polar and nonpolar fractions by silicic-acid and subfractionated by DEAE-cellulose-column chromatography. Free polar lipids substantially increased loaf volume; the increase was smaller when bound polar lipids were added. Lipid fractions isolated from various flours indicated no varietal differences. Total free lipids containing a mixture of nonpolar and polar components (in a ratio of 3:1) improved bread quality less than polar lipids alone. Nonpolar lipids decreased loaf volume and impaired crumb grain of bread baked from petroleum-ether-extracted flours; the deleterious effects were counteracted by polar lipids. The effects on bread depended on the levels and ratios of the nonpolar to polar lipids. Preliminary investigations were conducted on the effects on bread-making of adding separated fractions on a DEAE cellulose column. Fractions rich in galactosyl glycerides increased loaf volume most.

Tao and Pomeranz (1967) studied the effects of total, nonpolar, and polar wheat flour lipids on rheological properties of dough. The lipids were added to seven hard red winter wheat flours comparable as to milling extraction and protein contents but varying widely as to protein quality. Total (unfractionated) and nonpolar wheat flour lipids increased substantially the length of time needed to reach the point of optimum mobility in the Farinograph and in the Mixograph; polar lipids had little effect. The baking strength of a flour from which lipids had

been extracted had no effect on the contribution of the lipids to mixing characteristics. The effects of lipids on mixing increased with increase in level of added lipids. Nonpolar and total lipids exerted similar effects on untreated and on petroleum-ether-extracted flours. Temperature of peak hot-paste viscosity (as assessed by the Amylograph) was lowered about 4° by adding 2% flour lipids. Nonpolar lipids increased substantially peak viscosity; polar lipids had little effect. The results suggested that the principal effects on bread-making of adding flour lipids occur at the baking stage.

Lipid Binding in Wheat and in Bread-Making. Olcott and Mecham (1947) studied changes in lipid binding during dough mixing. A high-protein patent flour contained 1.5% total lipids, 70% of which were extractable with ether. The flour was mixed with water with a minimum of dough formation, and dried by lyophilization. Only 40% of the lipids were extractable with ether. After the flour was kneaded into a dough and dried, less than 10% of the lipids could be extracted. The capacity of the flour to "bind" lipids during wetting and dough formation was ascertained by determining the extractability of added flour lipids. At least three times the amount of lipid normally present could be bound by the dough making procedure. Phospholipids were bound preferentially. Most of the lipid bound was associated with the gluten, rather than with the nonprotein constituents of flour; and, when gluten was fractionated, the lipids were found to be bound to the "glutenin" rather than to the

gliadin fractions.

In a later study, Mecham and Weinstein (1952) reported on the effects of bread ingredients on lipid binding and the formation of lipoprotein during dough mixing. Salt decreased lipid binding in doughs, both of total lipid and of phospholipid, to 20 to 40% less than that occuring in the absence of salt. A "softener" of the polyoxyethylene stearate type had somewhat similar effects. Shortening (lard) appeared to decrease phospholipid binding slightly, but did not affect total lipid binding appreciably. Other bread ingredients had no detectable effects. The lipid contents of glutens washed out in salt solutions were lower than of those washed out in water, which parallels the observations on lipid binding in doughs. Baldwin et al., (1963) studied lipid binding in doughs and breads made by the conventional and the continuous processes. A blend of spring and winter wheats was used. Free (petroleum-ether-extractable) and bound (determined by acid hydrolysis following petroleum-ether extraction) lipids were extracted from flour, dough, and bread made by the conventional and the continuous processes. Fatty acids were fractionated by gas chromatography. The flour contained 2.3% fat, and dough and bread samples contained 5% fat (all on dry basis). More lipids were bound in continuous dough (49%) and bread (87%) than in conventional dough (34%) and bread (71%), and linoleic acid was preferentially bound.

In a later study, Baldwin et al., (1965) examined flour and

dough made by the continuous process. One portion of petroleum-ether-extracted bread was digested by amylases and the other portion was digested by proteases. After digestion, bread samples were re-extracted with petroleum ether. The bread contained 5% (dry basis fat), and 1% (20% total fat basis) was readily extractable with petroleum ether. About 1.2% (24% total fat basis) was released on enzymatic digestion of the starch, and 2.8% (56% total fat basis) on enzymatic digestion of the protein. On fractionation by thin-layer chromatography, mono-, di-, and triglycerides were found in the protein fraction along with phospholipids. Most of the free fatty acids and saturated monoglycerides were preferentially bound by the starch fraction. The unsaturated monoglycerides tend to be associated with the protein fraction. Diglycerides were bound to both starch and protein.

Daniels et al., (1966) reported the distribution of lipids in bread produced by conventional, Chorleywood, and continuous mixing methods. Free (petroleum-ether-extractable) and bound (extractable with a methanol/chloroform/water mixture following petroleum-ether extraction) lipids were extracted from flour, ingredients, dough, and bread prepared by the three mixing methods with 0.8% shortening in the formula. The free lipids (80% of the total) were made up of 40% from the flour, 32% from the added shortening and 8% from the soya flour. Bound lipids accounted for 20% of the total lipids present in the ingredients and arose almost exclusively from the flour. Lipid binding

increased during mixing in an ascending order of conventional, Chorleywood, and continuous methods. The increase in bound lipids during dough mixing was due mainly to nonselective binding of the available triglycerides. Bound lipids increased during dough mixing in the following order: conventional (32 to 45%), Chorleywood (37 to 52%), continuous (47.5 to 58%). Half of the polyunsaturated polar lipids were bound, which suggested that such lipids may be of much greater significance in understanding the behavior of bread in the oven than has been suspected.

Wootton (1966) used solvent extraction techniques in the studies of lipid binding and extractability. An Australian flour, Gabo variety, of 71% extraction was used. Dough and gluten were freeze-dried by lyophilization to less than 1% moisture content. Lipids of flour, dough, and gluten were extracted by 11 solvents. Weak polar solvents extracted up to 20% of lipids from gluten, 30% from dough, and 60% from flour. The most effective solvent used, ethanol : ether : water (2:2:1), extracted flour lipids almost quantitatively and about 75% of dough or gluten lipids. Nonpolar solvents extracted small amounts of nitrogen- and phosphorus-containing material from flour, and less from dough and gluten as a result of phospholipid binding. Ether and ethanol : ether : water were suggested as standard solvents to extract free and bound lipids when hydrolysate lipid was not determined.

Lipid binding decreased with increasing salt concentration

in the dough up to a concentration of 3.6% (on flour basis) and was unaltered by further addition of salt. A reduction in electrostatic phospholipid binding would be expected as a result of increasing the ionic strength of dough liquor, which would indicate that the ionic binding of phospholipid was disrupted.

Extraction efficiency and solvent properties were studied by using alcohol/halogenated hydrocarbon mixtures (1:1 v/v) and acetone/halogenated hydrocarbon mixtures (1:1 v/v). The former mixtures were shown to be the more efficient extractants of bound lipids. Extractions of gluten with pure solvents of differing dipole moment, dielectric constant, and molar volume were studied. The dipole moment of a solvent seems to bear no relation either to its efficiency in extracting lipids from gluten or to doughing ability. Increasing dielectric constant and low molar volume, in general, led to good lipid extraction properties. Addition of small amounts of water to the solvent increased lipid extraction.

Bloksma (1966) studied extraction of flour lipids by mixtures of butanol-1 and water. A flour was extracted stepwise with various butanol-1-water mixtures. With increasing water content from 0.2 to 25%, the extracted lipids increased from 1.16 to 1.37% and the extracted nonlipids increased from 0.06 to 0.27%. The results of an extensive study of the extraction of flour in columns with butanol-water mixtures, showed that optimum yield resulted when the column effluent contained 9% water in butanol.

MATERIALS AND METHODS

Sources of Wheat. All wheat samples were from the 1965 crop. Three hard red winter wheat samples from Fort Collins, Colorado, included the varieties Quivira-Tenmarq x Marquillo-Oro (C.I. 12995), Chiefkan x Tenmarq (Ks. 501097), and Chiefkan x Tenmarq (Ks. 501099). The three hard red spring wheat samples were from Eureka, South Dakota, and included Marquis (C.I. 3641), Lee (C.I. 12488), and Pilot (C.I. 11428). All wheats had large amounts of shrivelled kernels and were low in test weight (Tables 1 and 2). Each sample was separated into small, medium, and

Table 1. Description of wheat samples prior to separation into kernels of varying size.

Class and Variety	Test weight (lb/bushel)	Moisture (%)	Ash[a]	Protein[a] (N x 5.7) (%)
Hard red winter				
C.I. 12995	54.0	10.5	1.73	13.6
Ks. 501097	51.6	10.3	2.11	12.8
Ks. 501099	51.8	10.2	1.83	12.9
Hard red spring				
C.I. 3641	49.4	8.6	2.35	17.3
C.I. 12488	49.6	8.9	2.08	16.2
C.I. 11428	52.9	8.8	2.07	17.7

[a]On a 14% moisture basis.

Table 2. Separation of wheat samples into kernels of varying size by sieving.

Class and Variety	Small kernels (%, over 10 wire)	Medium kernels (%, over 9 wire)	Large kernels (%, over 8 wire)
Hard red winter			
C.I. 12995	10.0	46.9	33.3
Ks. 501097	5.9	35.5	47.2
Ks. 501099	8.7	44.7	40.9
Hard red spring			
C.I. 3641	21.5	65.4	9.8
C.I. 12488	14.6	71.5	12.2
C.I. 11428	7.3	74.4	16.9

large kernels by sieving for 3 minutes on a Ro-tap Testing Sieve Shaker (W. S. Tyler Co., Cleveland, Ohio). Broken kernels and impurities were removed from the small kernels by sieving through official grain dockage sieves (Burrows Equipment Co., Evanston, Illinois) and by hand separation.

For chemical analyses, the wheat samples were ground on a Hobart mill to pass a 40-mesh sieve.

Sources of Flour. Untreated flour was milled experimentally on an Allis mill from a composite grist of several hard winter wheat varieties grown in 1964 at a number of locations throughout the Great Plains. Expressed on a 14% moisture basis, the flour had an ash content of 0.42% and protein (N x 5.7) content of 13.1%. The bromate requirement of bread baked by a complete

formula was 3.0 mg per 100 g flour, and of bread baked without nonfat milk solids was 1.5 mg. Water absorption was 61.8%, and mixing time was 3½ min.

In addition, 16 flour samples milled from wheat from 1965 crop were used. Eight hard red winter and five hard red spring wheat samples were composited by variety from equal portions of wheat. Among the hard red winter wheats, Pawnee (C.I. 11669), Comanche (C.I. 11673), Quivira-Tenmarq x Marquillo-Oro (C.I. 12995), Chiefkan x Tenmarq (Ks. 501097), Chiefkan x Tenmarq (Ks. 501099), were from Clovis, New Mexico; Lincoln and North Platte, Nebraska; Fort Collins, Colorado; Stillwater, Cherokee, Goodwell, and Woodward, Oklahoma; Bushland and Chillicothe, Texas; and Garden City, Hays, Colby, and Manhattan, Kansas. Yogo (C.I. 8033) and Warrior (C.I. 13190) were from Hymore and Brookings, South Dakota; St. Paul and Crookston, Minnesota; and Huntley, Bozeman, Havre, and Moccasin, Montana. Karmont (C.I. 6700) was from Huntley, Bozeman, Sidney, and Havre, Montana. Hard red spring wheats, Thatcher (C.I. 10003), Selkirk (C.I. 13100), Marquis (C.I. 3641), Lee (C.I. 12488), and Pilot (C.I. 11428) were from Brookings, Eureka, Hymore, and Newell, South Dakota; Dickenson, Fargo, Minot, and Williston, North Dakota; Morris and Crookston, Minnesota; and Havre, Huntley, and Bozeman, Montana. Single samples of soft red winter, Seneca (C.I. 12529); durum, Wells (C.I. 13333); and white club wheat, Omar (C.I. 13072), were respectively obtained from agricultural experiment stations in Wooster, Ohio; Fargo, North Dakota; and Pullman, Washington.

The wheat samples were milled on a Miag "Multomat" mill. Certain chemical and bread-making analyses of the flour samples are given in Table 3.

Bread-Making. The basic bread formula included 100 g flour, 1.5 g salt, 2 g yeast, water as needed, and optimum potassium bromate. The basic formula was varied to include 3 g commercial vegetable shortening, 4 g nonfat dry milk solids, 6 g sucrose, and combinations of the optional ingredients. An optimum mixing time with the straight dough procedure and a 3-hr fermentation time at 30°C were employed. Punching and panning were performed mechanically. Baking time was 24 minutes at 218°C. Loaf volumes were measured with a National Manufacturing Co. volumeter, employing seed displacement. After the loaves had cooled, they were cut and their crumb grains evaluated. The following code was employed: S = satisfactory, Q = questionable, U = unsatisfactory.

Preparation of Dough and Yeast Samples. Samples of dough were removed at the end of mixing and of fermentation and frozen immediately on a block of dry ice. Yeast was similarly frozen. The frozen dough and baker's yeast were lyophilized. All samples were ground to pass 40-mesh sieve on a micro-Wiley mill.

Preparation of Crumb Samples. Samples of bread crumb were removed as soon as the loaves were cool, were frozen and lyophilized. All samples were ground to pass a 40-mesh sieve on a micro-Wiley mill.

Preparation of Water-Saturated 1-Butanol. Water-saturated

Table 3. Chemical compositions and bread-making characteristics of flours.

Class and Variety	C.I. No.	Ash[a] (%)	Protein[a] (%)	Baking absorption[a] (%)	Mixing time (min)	Bromate requirement (mg %)	Loaf volume (cc)	Crumb grain
Hard red winter								
Pawnee	11669	0.41	12.5	64.8	2 5/8	3.0	893	S
Comanche	11673	0.42	12.9	66.6	3 5/8	1.5	919	S
Qv-Tm x Mql-Oro	12995	0.42	12.5	66.4	5 1/8	1.0	922	S
Chiefkan x Tenmarq Ks.	501097	0.44	12.6	71.6	2 1/4	3.5	819	Q
Chiefkan x Tenmarq Ks.	501099	0.43	12.8	72.8	1 5/8	3.5	764	U
Yogo	8033	0.47	8.3	61.8	2 5/8	1.5	665	Q
Warrior	13190	0.47	8.7	61.0	3 5/8	0.5	670	S
Karmont	6700	0.44	8.4	64.0	3 1/4	2.0	693	Q-S
Hard red spring								
Thatcher	10003	0.54	13.2	66.6	3 1/4	2.0	920	S
Selkirk	13100	0.54	13.3	66.8	2 7/8	2.5	922	S
Marquis	3641	0.54	12.8	68.0	3	2.0	877	S
Lee	12488	0.54	13.3	65.4	3 1/2	1.5	913	S
Pilot	11428	0.52	12.7	55.4	3 1/8	1.0	900	S
Soft red winter								
Seneca	12529	0.30	8.7	67.2	3	1.0	740	Q-S
Durum								
Wells	13333	0.75	10.5	53.2	2 1/8	2.0	443	U
Soft white (club)								
Omar	13072	0.35	8.3	65.0	2 3/8	2.0	588	Q-U

[a] On 14% moisture basis.

1-butyl alcohol was prepared by mixing analytical grade 1-butyl alcohol with excess distilled water, and the upper layer was used after the mixture had stood for at least 24 hrs.

Lipid Extraction. Free lipids were extracted exhaustively with petroleum-ether (b.p. 35-60°C) in a Goldfish extractor. For re-extraction with water-saturated 1-butanol, residual petroleum-ether was allowed to evaporate over-night at room temperature.

Bound lipids were extracted, following petroleum-ether extraction, by treatment of the material twice with 100 ml water-saturated 1-butanol in a Stein mill (The Fred Stein Laboratories, Atchison, Kansas). The first extraction with 100 ml solvent was 3 times for 2 min. with 1 min. intervals; the second extraction 2 times for 2 min. The combined extracts were filtered and evaporated almost to dryness under vacuum in a glass apparatus at about 50°C. The extracts were kept under vacuum in a desiccator 40 hours over P_2O_5 at 4°C, and were extracted three times with Skellysolve F, and the combined upper layers were evaporated at about 50°C after centrifugation.

Total lipids were extracted with a chloroform-methanol-water mixture by the method of Bligh and Dyer (1959) as modified by Tsen et al., (1962). The solvent system used for the extraction was a mixture of 25 ml methyl alcohol, 12.5 ml chloroform, and water (including that present in the sample) to give a ratio of 2:1:0.8. Lipids in a 2 g sample were extracted in a micro-Waring Blendor (Ivan Sorvall, Inc., Norwalk, Conn., U. S. A.)

and homogenized for 2 min. To the mixture was then added 12.5 ml of chloroform; 12.5 ml water was added after 30 seconds blending; and the whole mixture was homogenized for an additional 30 seconds. The homogenate was centrifuged at 0°C at 10,000 x g for 10 min. Three layers were separated in the tube. The upper layer consisted of the methyl alcohol and water extract, the middle layer was a doughlike residue, and the bottom layer the chloroform extract. The upper layer was decanted. Most of the doughlike residue adhered to the tube, and the chloroform extract was easily transferred to a separatory funnel. The chloroform extract was then separated. An aliquot of the extract was filtered through Whatman No. 1 paper, and washed thoroughly with additional chloroform. The combined filtrate and washings were dried at 50°C to constant weight.

Results of lipid extractions are means of at least two determinations.

Thin-Layer Chromatography (TLC). Glass plates (20 x 20 cm) were coated with a 250-micron layer of silica gel G (E. Merck, A. G., Darmstadt, Germany) with a commercial spreader (C. A. Brinkmann Co., Great Neck, N. Y.). The plates were activated for 3 hours at 130°C and allowed to cool in a desiccator.

The extracted lipids were applied at a level of 100 μg per spot. The solvents used for one dimensional ascending development were: chloroform for nonpolar lipids, and a mixture of chloroform-methanol-water (65:25:4) (chloroform mixture) for polar lipids. All solvents were of analytical grade, redistilled

in glass. The spots were made visible by exposure to iodine vapor; by heating the plates for 25 min. at 180°C after spraying them lightly with a saturated solution of $K_2Cr_2O_7$ in 70% volume of aqueous-sulfuric acid (Blank et al., 1964); by spraying with a 0.2% solution of ninhydrin in butanol containing 1% pyridine (Lepage, 1964) as a spray specific for free amino acids; with a modified Dragendorff reagent (Mangold, 1961) for choline phosphatides and glycolipids; or with a molybdenum spray (Dittmer and Lester, 1964) for detection of phospholipids. Lipids separated by TLC were tentatively identified by use of specfic sprays and by comparing R_f values with those of pure compounds. Among the polar lipids, plant phosphatidyl choline, phosphatidyl ethanolamine, and phosphatidyl serine (from Applied Science Labs., State College, Pa.), and mono- and digalactosyl glycerides (gift from Dr. D. H. Hughes, Procter and Gamble Co., Cincinnati, Ohio) were used. Pictures were taken under UV light shortly after the plates were sprayed.

RESULTS AND DISCUSSION

The compositions of wheat kernels varying in size are shown in Tables 4 and 5. Consistent differences were found between hard red winter and hard red spring wheats. The results in Tables 4 and 5 are expressed on a weight basis. The hard red spring wheats contained higher ash and protein content than the hard red winter wheats. The average free lipids content in the hard red spring wheats was also higher than in the hard red winter wheats. Small differences were found in bound lipids in wheats from the two classes. The ash content decreased much and the protein content decreased little, with increase in kernel size. Compared on a kernel basis, the large kernels still contained about twice as much ash and protein as the small kernels. The small kernels contained, on a weight basis, substantially more free and slightly more bound lipids than the large kernels. Total lipid content per kernel depended more on kernel size than on wheat class or variety. In all samples, the large kernels contained about twice as much lipids as the small kernels.

Figure 1 shows a chromatograph of bound polar components in lipids from kernels of varying size, from spring wheat C.I. 3641. In addition to unidentified components with an R_f value of about 1.0, the main polar components are digalactosyl glyceride and phosphatidylcholine, smaller amounts of monogalactosyl glyceride, and relatively low concentrations of phosphatidyl ethanolamine, phosphatidyl serine, lysophosphatidyl choline, and several unidentified compounds. Visual observation showed no consistent

Table 4. Weight, ash, protein, and lipid contents (on a 14% moisture basis) of hard red winter wheat kernels of varying size.

Determination		C.I. 12995			Ks. 501097			Ks. 501099		
		Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
1000 Kernel wt.	(g)	11.2	18.0	23.3	10.5	17.9	26.2	10.8	17.0	22.8
Ash	(%)	2.03	1.77	1.64	2.25	2.01	1.93	1.99	1.89	1.83
Protein (Nx5.7)	(%)	13.7	13.5	13.7	13.2	12.7	12.7	13.2	12.7	12.8
Free lipids	(%)	2.10	1.95	1.80	1.62	1.46	1.41	1.84	1.78	1.67
Bound lipids	(%)	0.90	0.89	0.85	1.12	0.99	0.94	0.98	1.06	0.98
Free lipids as % of total		70.0	68.7	67.9	59.1	59.6	60.0	65.2	62.7	63.0
Total lipids per kernel (mg)		0.336	0.511	0.618	0.288	0.439	0.616	0.305	0.483	0.604

Table 5. Weight, ash, protein, and lipid contents (on a 14% moisture basis) of hard red spring wheat kernels of varying size.

Determination	C.I. 3641			C.I. 12488			C.I. 11428		
	Small	Medium	Large	Small	Medium	Large	Small	Medium	Large
1000 Kernel wt. (g)	9.6	13.6	20.8	10.6	14.0	21.4	11.9	18.0	23.8
Ash (%)	2.65	2.21	1.99	2.20	2.18	2.09	2.17	2.02	1.96
Proteins (Nx5.7) (%)	17.5	17.2	17.1	16.4	16.2	16.0	18.1	17.7	16.8
Free lipids (%)	2.00	1.96	1.83	1.82	1.74	1.67	2.24	2.08	1.77
Bound lipids (%)	1.02	0.96	0.88	1.01	0.98	0.97	1.02	0.92	0.82
Free lipids as % of total	66.2	67.1	67.5	64.3	64.0	63.3	68.7	69.3	68.3
Total lipids per kernel (mg)	0.290	0.397	0.564	0.300	0.381	0.565	0.388	0.540	0.616

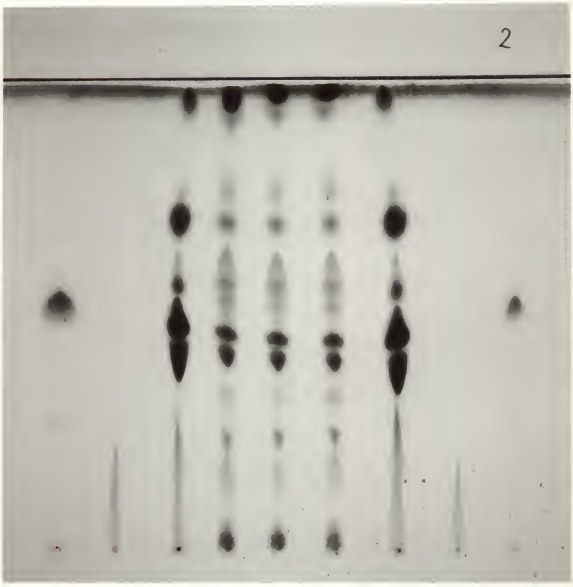

Fig. 1. TLC of bound polar lipids extracted with butanol following petroleum-ether from kernels of varying size of C.I. 3641 wheat. From left to right: phosphatidyl ethanolamine, phosphatidyl serine, mixture of 5 reference polar lipids, lipids from large, medium, and small wheat kernels, mixture of 5 reference polar lipids, phosphatidyl serine, and phosphatidyl ethanolamine. Reference lipids were applied at the 25 γ per spot level, wheat lipids at the 100 γ per spot level. Developed on TLC plates with chloroform : methanol : water (65:25:4); spots visualized by charring with sulfuric acid, picture taken under UV light. Tentatively identified as: a) Monogalactosyl glyceride, b) Phosphatidyl ethanolamine, c) Digalactosyl glyceride, d) Phosphatidyl choline, e) Lysophosphatidyl choline, f) Phosphatidyl serine.

or significant differences in the distribution of the polar components in lipids isolated from kernels of varying size.

No differences were found in the relative distribution of polar components in extracts of free and bound lipids from kernels of varying size (Fig. 2). There was, however, a considerable difference in the composition of polar lipids in the petroleum ether (free) and butanol-extractable (bound) lipids. Unlike the extract of bound lipids, the petroleum ether extract contained little digalactosyl glyceride and phosphatidyl choline, and practically no components with R_f values below 0.5 (primarily phosphatidyl serine and lysophosphatidyl choline). The petroleum ether extract was, however, relatively rich in mono-galactosyl glyceride and in an unidentified compound with an R_f value approaching 1.0.

The nonpolar and polar components in free lipids of kernels varying in size, from C.I. 12995 (HRW), C.I. 3641 (HRS), and C.I. 501099 (HRW) (Figs. 3 and 4). Figure 3 indicates that triglycerides are the main nonpolar component, and that all samples contained comparable amounts of free fatty acids and diglycerides. The hard red spring wheat samples seemed, however, to differ from the hard red winter wheat samples in R_f values of one of the monoglycerides, indicating probable variations in fatty acids.

Figure 5 shows the polar components in bound lipids (butanol extractable) from same samples as in Figs. 3 and 4. Comparing Figs. 4 and 5 confirms the large difference in the composition

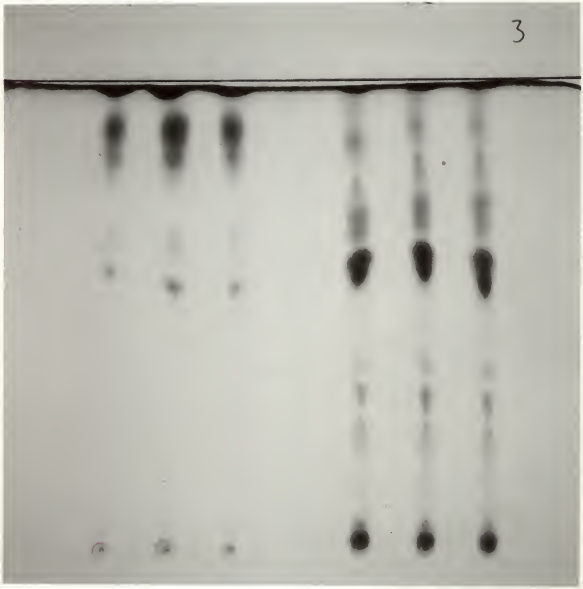

Fig. 2. TLC of free and bound polar lipids extracted from Marquis wheat kernels of varying size. From left to right: petroleum-ether extract of large, medium, and small kernels; butanol following petroleum-ether extract of large, medium, and small kernels; development and identification as in Fig. 1.

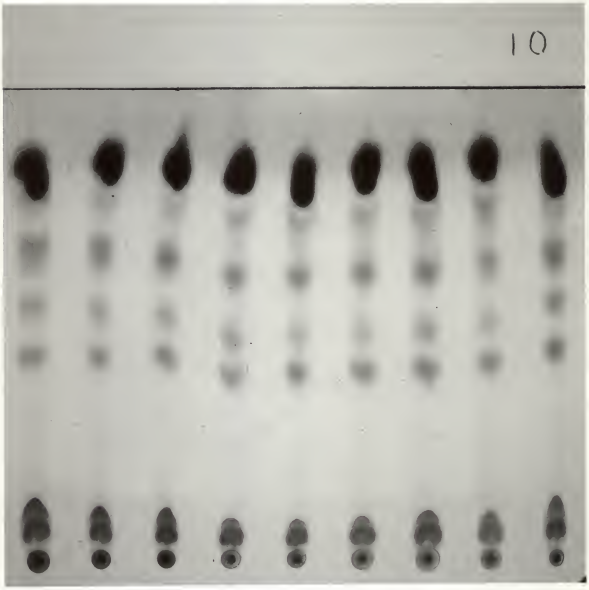

Fig. 3. TLC of nonpolar lipids in petroleum-ether extract of wheat. From left to right: from large, medium, and small kernels of C.I. 12995, C.I. 3641, and Ks. 501099. Developed with chloroform; other conditions as in Fig. 1. Tentatively identified as: a) Triglycerides, b) Free fatty acids, c) Diglycerides, d) Monoglycerides, e) Unfractionated polar lipids.

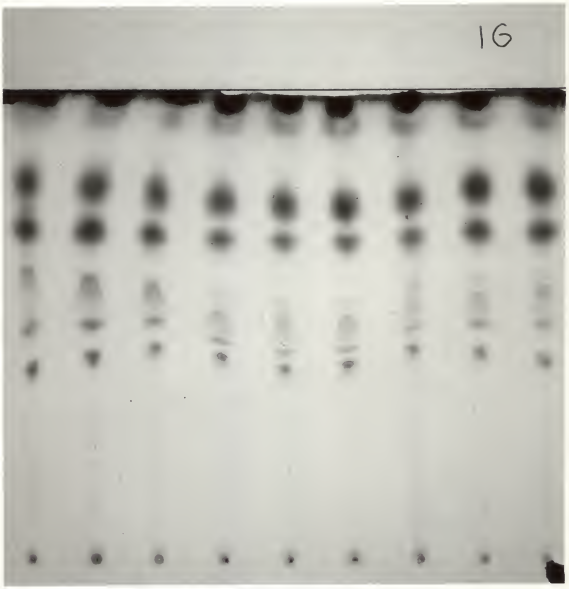

Fig. 4. TLC of polar lipids in petroleum extract of samples described in Fig. 3; development and identification as in Fig. 1.

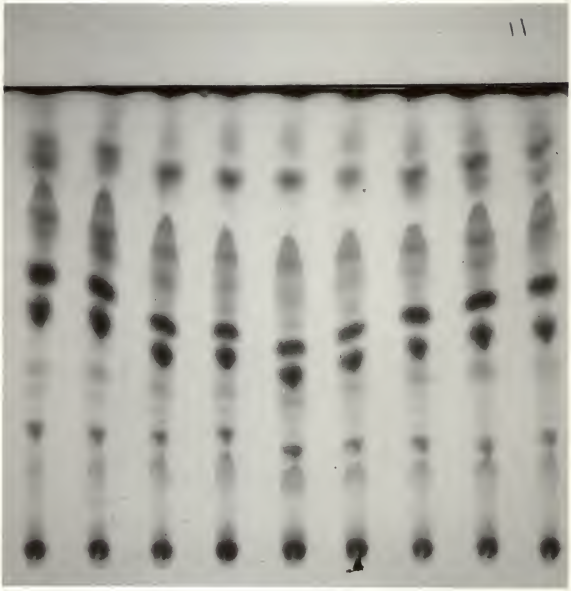

Fig. 5. TLC of polar lipids in butanol extracts following petroleum-ether in samples described in Fig. 3; developed and identified as in Fig. 1.

of polar lipids in the free and bound lipids, shown in Fig. 2.

Table 6 summarizes the effects of dough composition on bread quality. Effects of adding various combinations of shortening, milk solids, and sugar to the basic formula were tested. Crust color depended primarily on sugar content; the contribution to crust color of milk solids was small. Adding shortening to the basic dough had little effect; loaf volume was increased from 675 to 740 cc by adding shortening to a dough which contained 6 g sugar. The best bread was obtained from the complete dough formula (No. 6).

Table 6. Effects of dough composition on bread quality.

Dough composition	Proof height (cm)	Loaf volume (cc)	Crumb grain	Crust color
1) Basic[a]	below 5.8	345	U	Pale
2) Basic + shortening	below 5.8	355	U	Pale
3) Basic + milk solids	below 5.8	360	U	Light brown
4) Basic + sugar + shortening	6.5	740	Q-S	Brown
5) Basic + milk solids + sugar	7.1	695	Q	Rich brown
6) Basic + milk + shortening + sugar	7.0	765	Q-S	Rich brown
7) Basic + sugar	6.5	675	Q	Brown

[a]Flour, water, sodium chloride, yeast, and potassium bromate.

Table 7 shows the lipid contents of dough ingredients. Because of the relatively large amount of wheat flour and small percentages of milk solids and yeast in the dough formulation, wheat flour contributed much more than the other ingredients to the lipid contents of the dough or bread. However, shortening contributed a larger amount of lipids than wheat flour. The polar chloroform-methanol-water solvent system extracted much more lipids than petroleum ether. Bound lipids comprised 30% of the total flour lipids. This is in agreement with the findings of Daniels et al., (1966), but not in agreement with Baldwin et al., (1963), who reported that 62% of flour lipids were in a bound form.

Table 7. Lipid contents of dough ingredients.

Ingredient	Petroleum-ether extract (%)	Chloroform-methanol-water extract (%)
Flour	0.88	1.25
Nonfat dry milk solids	0.10	0.84
Yeast	0.58	3.44

Petroleum ether and chloroform-methanol-water extracts showed little difference in amounts and kinds of nonpolar lipids fractionated by TLC with chloroform (Fig. 6). Clearly shown is the presence of two kinds of triglycerides in milk lipids. Figure 7 shows petroleum ether extracted less polar lipids from

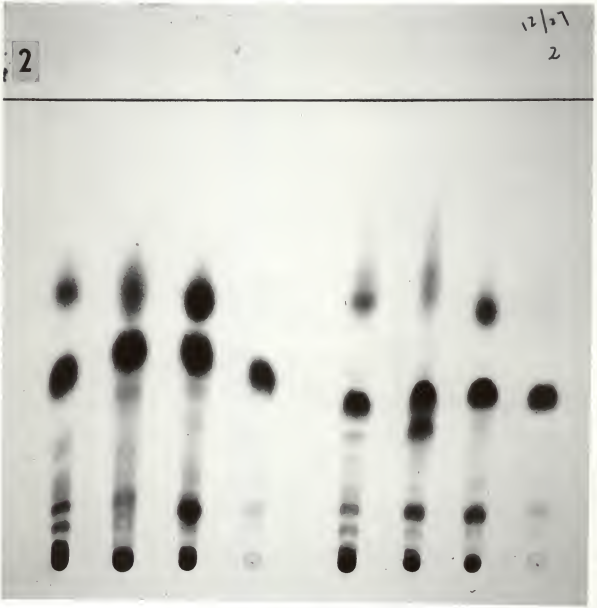

Fig. 6. TLC of lipids in dough ingredients. Samples 1-4 extracted with petroleum ether, 5-8 with chloroform-methanol-water. Samples 1 and 5, flour; 2 and 6, nonfat dry milk solids; 3 and 7, yeast; and 4 and 8, shortening lipids. Developed with chloroform; other conditions as in Fig. 1. Tentatively identified as: a) Triglycerides; b) Mono- and diglycerides; c) Unfractionated polar lipids.

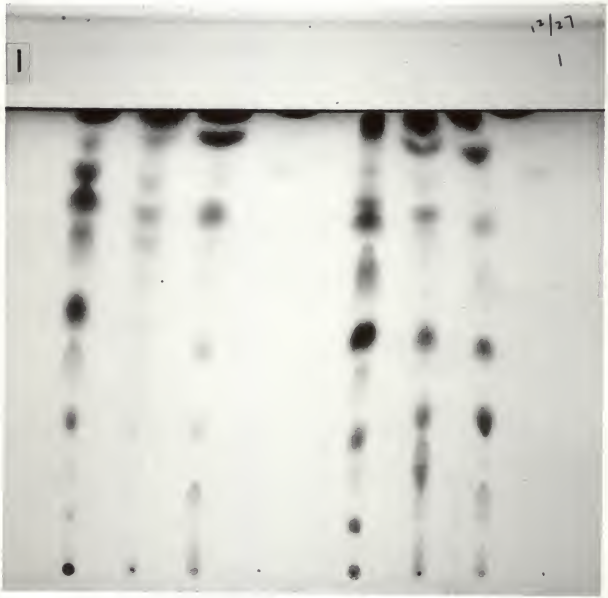

Fig. 7. TLC of lipids in dough ingredients. Samples as in Fig. 6 but developed with chloroform mixture. a) Nonpolar lipids; b) Monogalactosyl glyceride; c) Digalactosyl glyceride; d) Phosphatidyl choline.

flour, milk solids, and yeast than did chloroform-methanol. Only flour contained substantial amounts of polar components (phospholipids and glycolipids) both in a free and in a bound form. Glycolipids, mono- and digalactosyl glyceride, were present in relatively high concentrations in flour lipids only. Vegetable shortening contained mainly nonpolar lipids in both extracts.

The effects on the extractability of lipids of dough-mixing and fermentation, and of bread-baking are summarized in Table 8. Dough-mixing lowered the amounts of flour lipids soluble in petroleum ether to one-third the value found in untreated flour. Reduction in amounts of free lipids as a result of dough mixing indicates, therefore, partial binding of nonpolar flour lipids. There was no significant difference in amounts of petroleum-ether-soluble lipids in the dough between the beginning and the end of fermentation. Baking the fermented dough, however, reduced the amount of petroleum-ether-soluble flour lipids to half the amount in the dough, indicating additional binding of nonpolar flour lipids. Unlike the flour lipids, less than 15% of the shortening lipids were bound during dough-mixing and fermentation. At the baking stage, about 1/3-1/2 of the shortening lipids were inextractable in petroleum-ether.

Binding of flour or shortening lipids during mixing, fermentation, or baking seems to result from relatively weak bonding forces between lipids and flour proteins and carbohydrates. There was no consistent effect of dough-mixing, fermentation,

Table 8. Lipid contents[a] of dough and bread.

Dough composition	Petroleum-ether extract				Chloroform-methanol -water extract			
	Dough	Fermented dough	Bread crumb	Bread crust	Dough	Fermented dough	Bread crumb	Bread crust
Basic[b]	0.28	0.25	0.12	0.09	1.30	1.40	1.12	1.35
Basic + shortening	2.87	2.95	1.47	1.81	3.52	3.90	3.74	3.94
Basic + milk solids	0.20	0.16	0.09	0.09	1.35	1.31	1.33	1.33
Basic + sugar + shortening	2.66	2.83	1.44	1.79	3.51	3.50	3.60	3.81
Basic + milk solids + sugar	0.18	0.16	0.12	0.10	1.29	1.35	1.42	1.48
Basic + milk solids + shortening + sugar	2.57	2.82	1.45	1.62	3.60	3.55	3.58	3.68
Basic + sugar	0.29	0.30	0.13	0.18	1.20	1.23	1.37	1.33

[a]In lyophilized sample, %.

[b]Flour, water, sodium chloride, yeast, and potassium bromate.

or baking on the amounts of chloroform-methanol-extractable total lipids. Small differences are explicable by variations in moisture contents and relative concentrations of lipids in the dough.

Figure 8 shows TLC of petroleum-ether-soluble lipids in bread crumbs. The nonpolar lipids were fractionated with chloroform. Samples 2, 4, and 6, from bread baked with shortening, differ from the odd-numbered samples, baked without shortening. Consequently, in the crumb that contained shortening the relative amounts of flour lipids are reduced. TLC of chloroform-methanol-extracted lipids, fractionated by the chloroform mixture are shown in Fig. 9. The results in Fig. 9 show the same pattern as those in Fig. 8; higher relative concentrations of polar flour lipids were found in bread baked without shortening than in bread baked with shortening.

A comparison of Figs. 10 and 11 shows clearly that the polar wheat flour lipids are bound during mixing and baking of dough containing shortening, the nonpolar components (especially shortening lipids) are affected much less during processing of flour into bread.

Table 9 shows lipid contents of 16 flours which included 8 HRW, 5 HRS, one SRW, one Durum, and one club. The flours were all baked by the complete formula with shortening. The average total lipid content of the HRS flours (1.75%) was higher than that of the HRW flours (1.46%). Flour from durum wheat contained most free and total lipids. Soft red winter and club wheat

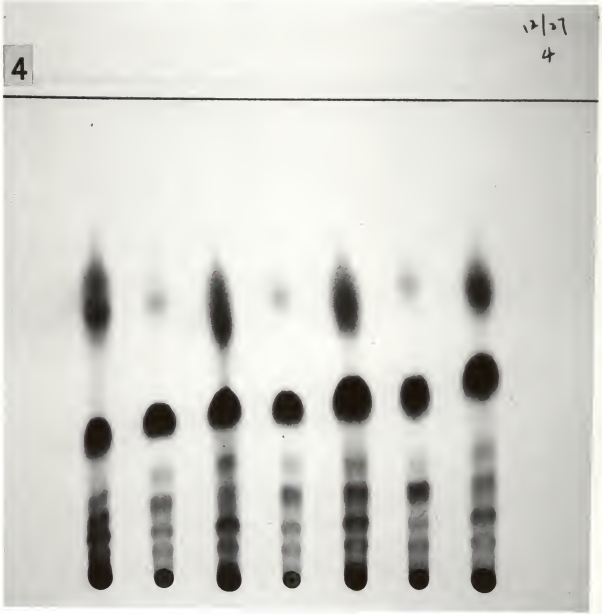

Fig. 8. TLC of petroleum-ether-soluble lipids in bread crumb. From left to right, from crumb of bread baked by formulae 1 to 7 (Table 6). Developed and identified as in Fig. 6.

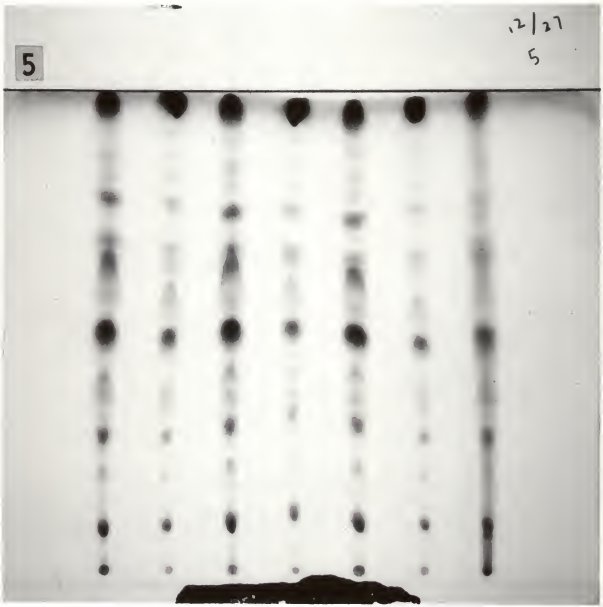

Fig. 9. TLC of chloroform-methanol-water-soluble lipids in bread crumb; samples as in Fig. 8; developed and identified as in Fig. 7.

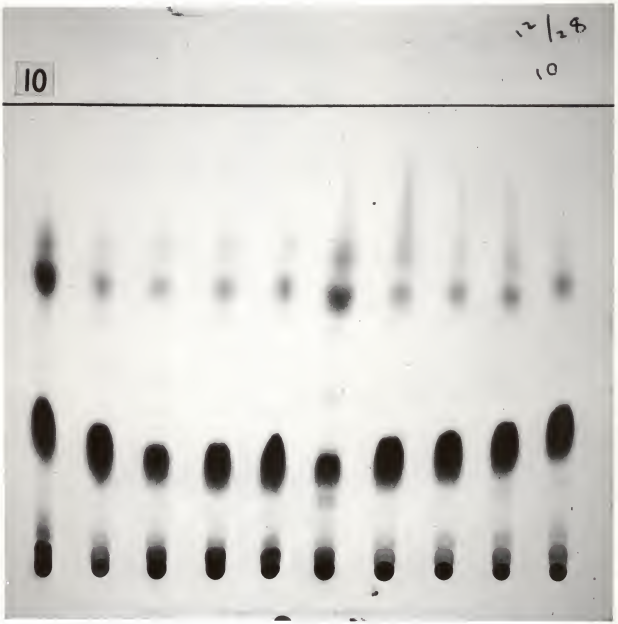

Fig. 10. TLC of lipids extracted from flour (1 and 6), complete dough formula (2 and 7), fermented dough (3 and 8), bread crumb (4 and 9), and crust (5 and 10). Samples 1-5 extracted with petroleum ether, 6-10 with chloroform-methanol-water. Developed and identified as in Fig. 6.

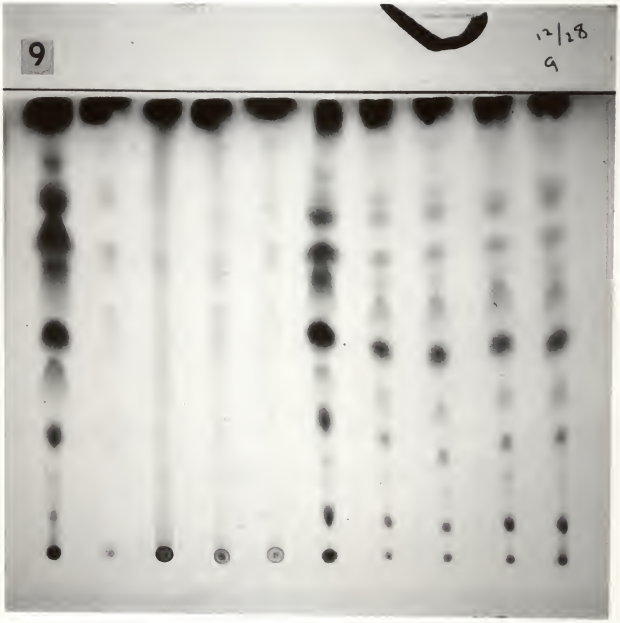

Fig. 11. TLC of lipids described in legend of Fig. 10; developed and identified as in Fig. 7.

Table 9. Lipid contents (on dry basis) of flour, dough, and crumb.

Class and Variety	Flour			Dough			Crumb		
	Free lipids (%)	Bound lipids (%)	Free lipids as % of total lipids	Free lipids (%)	Bound lipids (%)	Free lipids as % of total lipids	Free lipids (%)	Bound lipids (%)	Free lipids as % of total lipids
Hard red winter									
C.I. 11669	0.87	0.70	56.0	3.06	1.29	70.3	2.72	2.21	55.2
C.I. 11673	0.84	0.62	57.5	2.80	1.60	63.6	2.21	2.29	49.1
C.I. 12995	0.87	0.71	55.1	3.00	1.66	64.4	2.66	2.19	54.8
Ks. 501097	0.73	0.68	52.9	2.86	1.04	73.3	2.56	2.09	55.1
Ks. 501099	0.72	0.67	51.8	2.71	0.64	80.9	2.51	1.80	58.2
C.I. 8033	0.92	0.45	67.2	3.23	0.93	77.6	2.76	2.04	57.5
C.I. 13190	0.95	0.36	72.5	3.09	1.09	73.9	2.62	1.94	57.5
C.I. 6700	0.91	0.70	56.5	3.02	1.08	73.7	2.69	2.03	57.0
Hard red spring									
C.I. 10003	1.03	0.86	54.5	2.94	1.66	63.9	2.47	2.83	48.4
C.I. 13100	0.98	0.75	56.6	2.81	1.50	65.2	2.38	2.65	47.3
C.I. 3641	1.06	0.64	62.4	2.87	1.68	63.1	2.74	2.56	51.7
C.I. 12488	1.06	0.52	67.1	3.13	1.39	69.2	2.70	2.29	54.1
C.I. 11428	1.13	0.51	68.9	2.92	1.45	63.6	2.72	2.16	55.7
Soft red winter									
C.I. 12529	0.55	0.63	46.6	3.16	1.11	74.0	2.82	1.84	60.5
Durum									
C.I. 13333	1.27	0.67	65.5	3.23	1.42	69.5	3.12	1.94	61.7
Soft white (club)									
C.I. 13072	0.89	0.63	58.6	3.31	0.93	78.1	3.09	1.48	67.6

flours contained less ash and protein, and were also lower in total lipid contents than hard wheat flours. The result suggested possible correlations between lipids contents and ash or protein. But, the correlations between ash and protein (r = 0.27), bound lipids and protein (r = 0.41), and free lipids and protein (0.04) were low, and statistically insignificant. It seems, therefore, that lipid binding is affected by additional factors, i.e. protein quality.

After baking, total lipid contents of crumbs (4.81% total average) were higher than of the doughs (4.30% total average). During baking increase in bound lipids (0.49 to 1.16%, average 0.84%) was substantially higher than decrease in free lipids (0.11 to 0.59%, average 0.34%). The higher average level of total lipids in bread crumb than in dough resulted partly at least from using small amounts of shortening lipids (0.15 to 0.25 g) in dough handling following mixing and in pan coating. In addition, however, some unexplained changes took place. Daniels et al., (1966) reported unexplained losses in lipids during baking. Despite large differences of the studied flour (Table 3), no consistant varietal effects on lipid binding during dough mixing or baking were recorded. The results are complicated by the tested flours varying both in protein contents and protein quality.

Detailed examinations by TLC were made on lipids isolated from Comanche (HRW), Marquis (HRS), Seneca (SRW), Wells (Durum), and Omar (Club). No significant or consistent differences were

found in TLC pattern of nonpolar or polar flour lipids of the flours. Previously observed differences between polar free and polar bound lipids were confirmed. Again, only trace amounts of polar lipids could be extracted with petroleum ether from doughs; the polar lipids in dough were almost entirely present in a bound form. Both in bound lipids of flour and dough, digalactosyl glyceride was present at a lower concentration in lipids from Marquis variety, and especially in Wells variety than in lipids from the other varieties. Additional, more comprehensive tests should be conducted to confirm the results from single samples.

Nonpolar lipids extracted from dough with petroleum ether and with water-saturated butanol following petroleum ether are compared in Fig. 12. Most of the nonpolar lipids in the dough were free. However, relatively substantial amounts of nonpolar lipids (mainly triglycerides) could be extracted only with water-saturated butanol, which confirms the view that partial binding of nonpolar lipids takes place during dough mixing.

Some differences in patterns of free nonpolar and bound polar lipids from dough and crumb are indicated in Fig. 13 and 14. The differences might be related to modifications during baking and suggest need for additional studies on interaction between lipids and other wheat flour components.

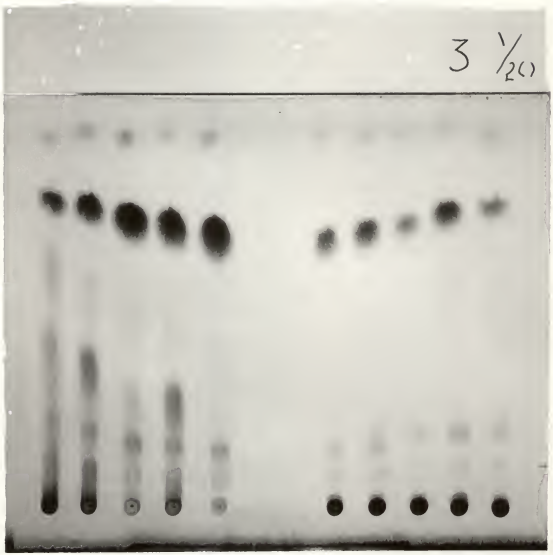

Fig. 12. TLC of free and bound lipids in doughs. Samples 1-5 extracted with petroleum ether, 6-10 with water-saturated butanol following petroleum ether. Samples 1 and 6, from Comanche; 2 and 7, from Marquis; 3 and 8, from Seneca; 4 and 9, from Wells; 5 and 10 from Omar wheat. Developed and identified as in Fig. 6.

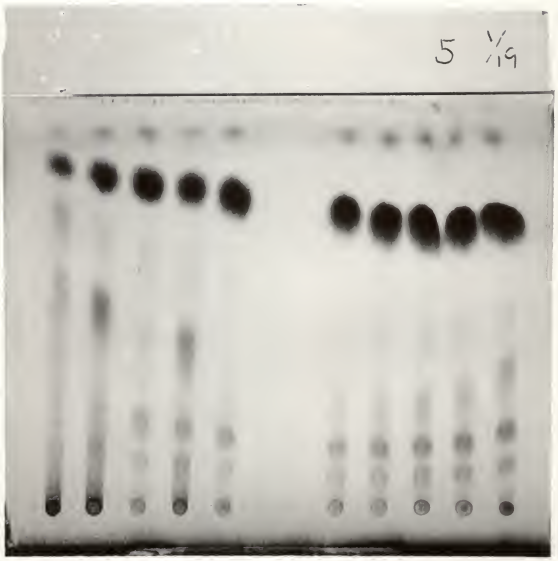

Fig. 13. TLC of free nonpolar lipids in dough and crumb. Source of samples as in Fig. 12, but 1 to 5 were extracted from doughs and 6 to 10 extracted from crumbs with petroleum ether. Developed and identified as in Fig. 6.

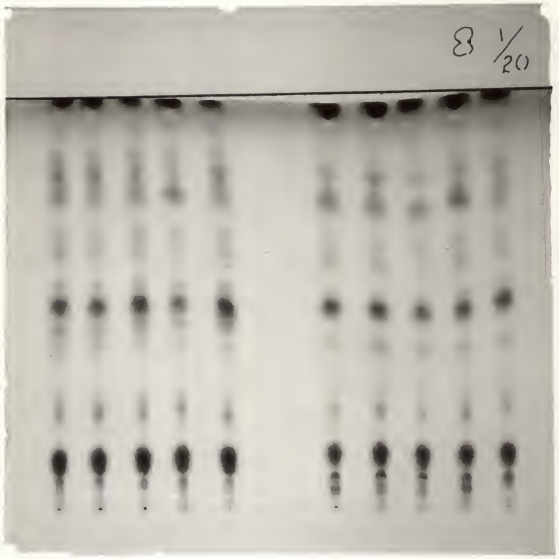

Fig. 14. TLC of bound polar lipids in dough and crumb. Samples from same wheat varieties as in Fig. 13, but extracted by water-saturated butanol following petroleum ether. Developed and identified as in Fig. 7.

SUMMARY

Wheat samples from three hard red winter and three hard red spring varieties were separated into kernels of varying size. Concentration of free lipids (petroleum-ether-extractable) and of bound lipids (extracted by water-saturated butanol following extractions with petroleum-ether) were higher in small than in large kernels. Small kernels contained, on a weight basis, substantially more free and slightly more bound lipids than the large kernels. Thin-layer chromatography showed triglyceride as the major nonpolar component, and digalactosyl glyceride and phosphatidyl choline as the major polar components. Concentrations of individual nonpolar or polar components were not affected significantly by kernel size.

Free and total (extracted by chloroform-methanol-water mixture) lipids were extracted from flour, dough, fermented dough, bread crumb, and bread crust. Dough formulations used in breadmaking included, in addition to a basic formula of flour, water, yeast, and sodium chloride, either sugar, commercial vegetable shortening, and dry milk solids, or their combinations. Petroleum-ether-soluble flour lipids were reduced to one-third the original amount during dough mixing or fermentation; subsequent baking lowered the residual free lipids to half (of dough or fermented dough). Processing flour into bread had no effect on the amount of total lipids extractable by the chloroform-methanol-water mixture. Fractionation of extracted lipids by TLC showed that much more polar wheat flour lipids than nonpolar were bound

during dough mixing.

Free and bound lipids were extracted from 16 flours which included 8 HRW, 5 HRS, one SRW, one Durum, and one Club. They were all baked by a complete formula with shortening. Average amounts of free and bound lipids were higher in HRS than in HRW wheat varieties. Distribution of polar lipids in petroleum ether extracts varied consistently from distribution of polar lipids in butanol extracts. Only trace amounts of polar lipids could be extracted with petroleum ether from doughs. Relatively substantial amounts of nonpolar lipids could be extracted from doughs with water-saturated butanol, confirming partial binding of nonpolar lipids during dough mixing. Comparison by TLC of lipids in flours, doughs, and crumbs of five wheat varieties showed some differences in patterns of free nonpolar and bound polar lipids.

SUGGESTIONS FOR FUTURE WORK

Wheat flour lipids are a complex mixture of known and unknown components. Studies of a heterogenous mixture of lipids and of their effects on bread-making are of limited value. Much useful information could be obtained if individual lipids were isolated in pure and unmodified form and compared with lipids obtained by synthesis in vitro or biosynthesis. Once pure fractions are obtained, their interaction could be evaluated in model systems with proteins, and in actual bread-making.

Protein content of wheat is an important index of bread-making potential. Protein contents and quality might feasibly govern lipid binding in wheat flour and in bread-making. Relation of lipids and protein quality of wheat flour has been studied little. The effects of lipid binding by various flour components should be studied by varying the amounts of protein from a single source in dough, and by comparing the effects of lipids on doughs baked from flours comparable in protein contents and varying in protein quality.

This study indicated possible differences in composition of polar lipids in various flours and in modification of the polar components during the baking stage. More extensive investigations are needed to confirm and substantiate the preliminary findings.

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LIPID BINDING IN WHEATS AND IN FLOURS VARYING WIDELY IN BREAD-MAKING POTENTIALITIES

by

CHIEN-MEI CHIU

B. Sc., Tunghai University (Taiwan), 1963

AN ABSTRACT OF A MASTER'S THESIS

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Department of Grain Science and Industry

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Lipid binding in dough has been studied by many investigators. Literature review indicates the changes in extractability of lipids during bread-making might be related to bread-making quality.

Wheat samples from three hard red winter and three hard red spring varieties were separated into kernels of varying size. Concentration of free lipids (petroleum-ether-extractable) and of bound lipids (extracted by water-saturated butanol following extraction by petroleum-ether) were higher in small than in large kernels. Small kernels contained, on a weight basis, substantially more free and slightly more bound lipids than the large kernels. Thin-layer chromatography showed triglycerides as the major nonpolar component, and digalactosyl glyceride and phosphatidyl choline as the major polar components. Concentrations of individual nonpolar or polar components were not affected significantly by kernel size.

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